Ultrasensitive in Vivo Fluorescence Imaging of Mesenchymal Stem Cells and Small Molecule-Induced Pluripotent Stem Cells in BALB/c mice.

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Research

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Abstract

Background

Mesenchymal cell has been frequently used in clinical studies. Mesenchymal stem cells (MSCs) are self-renewing, multipotent stem cells with the potential to differentiate into multiple mesoderm lineages. But MSC have limitation in clinical application for treating human diseases because they can differentiate several types of cell but not all types. PSL (Pluripotent Stem cell Like cell) are newly developed pluripotent stem cells from human mesenchymal stem cells (hMSC) induced by small molecule compounds. These cells have potential advantages for clinical cell treatment compared with ESCs and iPSCs.

Methods

We induced pluripotency from MSC using small molecules. It has tried to trace MSC and PSL in mice using bioluminescent techniques, which can detect visible light emitted from cells labeled with miRNA conjugated fluorescent molecules.

Results

MSCs predominantly migrate into the brain and testis. They also migrate to the liver, omentum, mesentery, kidneys and spleen. Migration of PSL is similar to MSCs, in that they go to the brain, testis and other intraperitoneal organs. Fluorescent images of explanted organs show that the intensity of brain images is higher in the PSL mouse group than the MSC mouse group. However, testis, image intensity is higher in MSC mouse group than the PSL mouse group. In PSL but not MSC mice, fluorescence persisted at the injection site in the tail.

Conclusions

In this study, injected MSCs and PSL predominantly migrated to the brain and testis. But, PSL migration was more than MSC migration in Brain. Both cell types had a similar migration pattern except for persistent fluorescence at injection site in the tail vein of PSL mice. We expect these cell therapy may have many potentials for clinical studies on these notable treatments.

Background

Stem cell-based therapies are being actively explored as a potentially innovative therapeutic strategy for various genetic and acquired diseases.

Recently, the reprogramming of human somatic cells into human-induced pluripotent stem cells (hiPSCs) has been reported. As hiPSCs can differentiate into all cell lineages this has opened new opportunities for damaged organ repair[1,2]. Although many hiPSCs clinical studies have been carried out on various disease, there are only a few reports that it has been effective at curing disease. One problem is that

hiPSCs are made with genetic mutations which may have tumorigenic potential[3]. Another problem is the low yield of hiPSCs, as it is important to administer enough stem cells to be effective.

We have recently developed pluripotent stem cells from umbilical cord, adipose tissue, cord blood and bone marrow MSCs. We named these pluripotent stem cells as PSL (U.S. Patent No. 10,131,881., 2018).

PSL (Pluripotent Stem cell Like cell) are pluripotent stem cells induced from human mesenchymal stem cells (hMSC) by the addition of the small molecule compounds. This is a novel approach for inducing pluripotency. PSL are pluripotent stem cells like as ES and iPSCs. The advantage of PSL compared to other hiPSCs is that these cells have no mutational potential and can be produced with a high yield rate. Recently, there has been a report on chemically induced pluripotent stem cells (CiPSCs) which also can be induced by small molecule compounds[4]. The CiPSCs in this report originate from mice, while the PSL in this study are the first chemically induced human pluripotent stem cells developed from human cells. Mesenchymal cell has been frequently used in clinical studies. Mesenchymal stem cells (MSCs) are self-renewing, multipotent stem cells with the potential to differentiate into multiple mesoderm lineages[5,6,7,8]. MSCs are non-immunogenic[9] and regarded as a promising source for cell-based treatments of various complicated disease, such as cardio cerebrovascular disease[10,11], neuronal injury[12,13], hepatic diseases[14] and respiratory disease[15]. And another character of MSCs is their immunosuppressive effect in graft-versus-host disease[16,17]. The pathological mechanisms of MSCs have been well studied *in vitro*. However, *in vivo* studies are still rare and the fate and survival of MSCs *in vivo* are not well understood. In contrast, the use of iPSCs has rarely been reported in clinical trials. To know the exact physiological mechanisms of MSCs or PSL, tracking and localization of these cells *in vivo* is necessary. If these cells can be tracked, the healing and reconstructive process can be studied.

Fluorescence Imaging (FI) methods can detect visible light emitted from cells labeled with miRNA conjugated fluorescence molecules[18,19,20]. FI can track cells implanted in living animal non- invasively and in real time[21]. This technology has been used in this study to trace and localize MSCs and PSL.

Using this technique, gross photos were obtained to localize the accumulation of these cells *in vivo*. The animals were sacrificed and specimens extracted to provide localized fluorescent images from organs including the brain, liver, heart, spleen, kidney, testis, lung, adipose tissue, omentum and mesentery.

There have been a few reports on MSC localization and tracing after administration[22,23]. This study on the localization of PSL and MSCs may be useful for future applications in clinical trials.

Methods

Preparation of MSCs

Human umbilical cords were obtained from full-term Caesarean section deliveries and then stored in Dulbecco's modified Eagle medium (DMEM)/F12 (1:1) supplemented with 100 U/ml penicillin and 100

µg/ml streptomycin (GIBCO, Invitrogen Inc. Carlsbad, CA, USA). To isolate stem cells, the cord was cut into 4–5 cm long pieces, and the vessels were pulled away to leave only Wharton's Jelly (WJ). WJ was cut into 1–2-mm3 pieces and digested with 1 mg/ml of collagenase type 1 (Millipore Sigma, St. Louis, MO, USA) in phosphate-buffered saline (PBS) at 37 °C for 30 min. The digested mixture was then passed through a 100-µm filter (BD Biosciences, Franklin Lakes, NJ, USA) to obtain cell suspensions. The cells were washed with PBS solution and then cultured in DMEM/F12 medium containing 10 % fetal bovine serum, 2 mmol/L glutamine, 1 % nonessential amino acids, and 1 % penicillin/streptomycin (GIBCO, Invitrogen Inc., Carlsbad, CA, USA) at 37 °C and 5 % CO_2. Non adherent cells were removed when the medium was changed on day 3. The multipotent MSCs, which were adhering to the plastic bottom were then collected. The multipotent MSCs phenotype is defined as the co-expression of antigens (CD105, CD73, and CD90 [≥95 % positive]) and the absence of hematopoietic lineage markers (CD45 and CD34 [≤2 % positive]). In summary, multipotent MSCs phenotype was confirmed by positive expression markers (CD105 CD73 and CD90-FITC) and negative expression markers (CD45 and CD34-FITC).

Induction of Adipogenic and Chondrogenic Differentiation in MSCs

To induce chondrogenic differentiation, MSCs were treated with chondrogenic medium. The medium was changed twice a week. The chondrogenic medium consisted of DMEM-F12 supplemented with 0.1 µM dexamethasone, 50 µg/mL AsA, 100 µg/mL sodium pyruvate (Sigma-Aldrich), 40 µg/mL proline (Sigma-Aldrich), 10 ng/mL TGF-β1, and 50 mg/mL ITS+ premix (Becton Dickinson; 6.25 µg/mL insulin, 6.25 µg/mL transferrin, 6.25 ng/mL selenius acid, 1.25 mg/mL bovine serum albumin and 5.35 mg/mL linoleic acid).

To induce adipogenic differentiation, the MSCs were incubated in adipogenic medium which was changed twice a week. The adipogenic medium consisted of DMEM-F12 supplemented with 0.5 mM 3-isobutyl-1-methylxanthine (IBMX; Sigma- Aldrich), 1 µM hydrocortisone (Sigma-Aldrich), 0.1 mM indomethacin (Sigma-Aldrich), and 10% FBS.

Preparation of PSL Cells from MSCs

We used small molecular compounds (STC-F002), extracted from sea algae (Eklonia cava). The MSCs was cultured in DMEM-F12 medium containing 10% FBS. When 90% of the dish bottom was covered with these cells, approximately day 5, 10ug/mL of STC-F002 was added to the culture medium (U.S. Patent No. 10,131,881(2018)).

Approximately 10 days later, distinct types of colonies appeared which looked similar to human Embryonic Stem cell (hES) colonies. The hES cell like colonies were carefully picked and mechanically disaggregated into small clumps.

Expression of Pluripotent Stem Cell Markers in PSL

The PSL cells strongly expressed all the characteristic pluripotent stem cell markers. These included SSEA4, a cell-surface glycosphingolipid used for detecting human ES cells; Oct3/4, a protein involved in the self-renewal of human ES cells, Sox2, a transcription factor that controls genes involved in embryonic development and AP, ubiquitous membrane-bound glycoprotein for detecting pluripotent stem cell[40]. In comparison MSCs were either negative or weakly positive for the pluripotent stem cell markers.

Primary antibodies used for IH of SSEA4 (1:100, Developmental Studies Hybridoma Bank), OCT4 (1:200, R&D Systems), SOX2 (1:200, R&D Systems). The secondary antibodies were Alexa488-conjugated goat anti-rabbit IgG (1:500, Invitrogen). The nuclei were stained with 1 μg/ml Hoechst 33342 (Invitrogen).

Induction of Osteogenic, Chondrogenic, Hepatogenic and Neurogenic Differentiation in PSL

To induce osteogenic differentiation, fifth- to seventh-passage umbilical cord derived stem cells were treated with small molecules for 10 days. The PSL were then cultured in osteogenic medium for 1 week with medium changes twice a week. Osteogenic medium consists of DMEM-F12 supplemented with 0.1 μM dexamethasone (Sigma-Aldrich), 10 mM β- glycerol phosphate (Sigma-Aldrich), and 0.2 mM ascorbic acid (AsA; Sigma-Aldrich). Von kossa staining was used for osteogenic differentiation

To induce chondrogenic differentiation, PSL cells were cultured in chondrogenic medium for 2 weeks. Medium changes were carried out twice a week. Chondrogenic medium consisted of DMEM-F12 supplemented with 0.1 μM dexamethasone, 50 μg/mL AsA, 100 μg/mL sodium pyruvate (Sigma-Aldrich), 40 μg/mL proline (Sigma-Aldrich), 10 ng/mL TGF-β1, and 50 mg/mL ITS+ premix (Becton Dickinson; 6.25 μg/mL insulin, 6.25 μg/mL transferrin, 6.25 ng/mL selenous acid, 1.25 mg/mL bovine serum albumin (BSA), and 5.35 mg/mL linoleic acid). Alcian blue was used to stain for chondrogenic differentiation

To induce hepatogenic differentiation, PSL cells were cultured in hepatogenic medium, which was changed twice a week. Hepatogenic medium consisted of IMDM supplemented with 10nM dexamethasone, 100ng/ml HGF, 50ng/ml FGF and 5.5 ug/mL ITS+ premix. Hepatogenesis was assessed by fluorescence imaging (alpha-fetroprotein)

To induce neurogenic differentiation, a multistep protocol was used. PSL cells were cultured in step 1 medium for 7 days, and then step 2 medium for 7 days. Step 1 medium consisted of DMEM-F12 supplemented with 2% B27 supplement, 2mM L- glutamin, 30ng/ml EGF, and 25ng/ml bFGF. Step 2 medium consisted of DMEM-F12 supplemented with 2% FBS and 25ng/ml BDNF. Neurogenesis was assessed on day 6 and day 10, and imaging for nestin was performed on day 12.

Injection of MSCs and PSL

BALB/c mice (6–8 weeks old) were provided by Oriental Bio Inc. (Seongnam, Korea) and were allowed to adapt to the new environment for 7 days. Experiments were performed in accordance with the Institutional Guidelines for the Care and Use of Laboratory Animals in Research and Use Committee of

Bundang Hospital of Seoul National University, Korea. This study was approved by the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee (BA1109-091/064-01)[37].

The mice were divided into an MSCs (n=9) and an PSL (n=9) group. MSCs and PSL cells were injected into the tail veins of the mice in each group. 5x105 MSCs and 5x105 PSL cells were administered per mouse. In addition, a control group was used for the base line.

Preparation of the miRNA-221 molecular beacon

A miRNA- 221 molecular beacon was used to bio-image the MSCs and PSL cells.

To construct the miRNA-221 MB, NH2-modified molecular beacon, miRNA-221 oligos (5'-ATGAACAGACGCCCCAAACCGACCCAAAG-BHQ2-3') were synthesized by Bioneer Inc. (Daejeon, Korea). MBs with complementary sequences (miRNA-221) were also synthesized. The Cy5.5-COOH fluorophore were purchased from molecular probe (Thermo Fisher, Waltham, USA). The miRNA-221-linked MB contains cy5.5 (excitation/emission wavelength: 595/625 nm) and BHQ2.

Synthesized miR-221 MB probe was confirmed with selectivity assay by fluorescent spectrometry[37].

The miR-221 MB probe was incubated with the MSCs and PSL cells check the expression of intracellular miR-221 in a tube for 5 min at room temperature. miR-221 MB was also able to bind to miR-221 in the human MSCs and PSL cells from different pathological origins.

In vivo imaging

MSCs and PSL cells were traced *in vivo* with the miRNA conjugated fluorescent dye. The miRNA conjugated fluorescent dye system was conducted and transferred to MSCs and PSL cells. Mice were anesthetized before MSCs and PSL cells were injected. Cell transplantation was conducted as described above. miRNA-221 was tracked from approximately 30 min after miRNA221-labeled MSCs and PSL cell injection. Near-infrared (NIR) fluorescence images (595/625 nm) of the mice were taken using an IVIS Spectrum 2D *in vivo* imaging system (Perkin Elmer, Connecticut, USA) to monitor miRNA-221[37]. cell tracing was performed at about 10 min after substrate injection.

Imaging analysis were normalized mice imaging with MB probe injection to eliminate signal interference using mice imaging without cell injection

Immunohistochemical analysis of animal tissues

Samples from each organ were snap-frozen in OCT compounds (Sakura). Cryosections (5–15 μm) were air-dried and then fixed in 4% paraformaldehyde (Seongnam, Bioseasang, Korea) for 10 min.

After blocking with 5% (v/v) goat serum (Life Technologies) for 1 h at room temperature, the samples were incubated with the primary SSEA4 antibody (Santacruz, USA) for 12h at 4 °C. After washing in PBS, they were stained with the secondary antibody and H33342 (Life Technology, Eugene, USA) for 30 min at

room temperature. Immune staining was observed and recorded using a Zeiss LSM 800 confocal laser microscope with a 20x NA 0.6 objective (Carl Zeiss Microscopy GmbH). The Emission range of the lasers was 405 nm for H33342 and 625 nm for miRNA-221-MB. The microscopy image data was reconstructed using a maximum intensity projection algorithm implemented in the Zeiss ZEN lite blue 2.5 software (Carl Zeiss Microscopy GmbH).

Results

Characterization of MSCs

MSCs cells were highly positive for CD105, CD73, and CD90 but negative for CD45 and CD34 (Figures 1A)[24]. To complete the characterization, the cells were cultured in differentiation media triggering either adipogenesis or chondrogenesis. Adipogenic induction was demonstrated by the lipid dye Oil Red O (Figures 1B). Chondrogenic induction was monitored by coloring acidic mucins, and type II collagen with Alcian Blue (Figures 1B).

PSL induction by small molecules

MSCs cultured with small molecules (STC-F002) for 7–10 days formed compact colonies that were positive for Oct3/4, SOX2 SSEA4 and AP (Alkaline phosphatase) expression (Figures 2A, C). Both the PSL colonies and individual PSL cells strongly expressed all of the characteristic pluripotent stem cell markers that were examined. These included Oct3/4, Sox2, SSEA4 and AP. MSCs derived from Wharton's jelly tissue were either negative or weakly positive for these pluripotent stem cell markers

To complete our examination of the potential for spontaneous differentiation into the three germ lines, PSL cells were differentiated into mesodermal lineages using chondgenic and osteogenic differentiation medium. Formation of chondrocytes was detected using alcialn blue and formation of ostreoblasts was detected using Von Kossa stain. (Figures 2B)[25]. PSL have also shown the ability to differentiate into neuronal cells lineages. Neural-like cells were detected by immunofluorescence using anti-human nestin antibodies. PSL cells showed significant expression of nestin as shown by H33342 staining (Figures 2B), confirming their potential to spontaneously differentiate into ectodermal cells, unlike the control MSCs which were negative for nestin. PSL cells were differentiated into endodermal cells by exposing them to hepatocyte differentiation medium for 7 days. The differentiation of PSL cells were confirmed by alpha-fetoprotein, which is expressed during the development of the endoderm and by the progenitors of hepatocytes(Figures 2B). These results demonstrate that PSL are pluripotent stem cells capable of differentiation to induced ectodermal, mesodermal and endodermal cells in culture.

Gross fluorescent *in vivo* images of MSCs and PSL

Early fluorescent images of both groups show the accumulation of cells around the pleural and lung area of the prone photo. They gradually spread to other areas and become fainter over time (Figures 3A, D). Fluorescence images show accumulation into the peritoneal area and which persists until the animals were sacrificed in the supine photo. The peritoneal area may include the liver, omentum, mesentery, kidneys and spleen.

Fluorescence was also seen in brain area in both groups. The intensity of fluorescence in the brain was higher in the PSL group. A fluorescence signal at injection site in the tail persisted for the entire experiment (9 day) in PSL mice. In contrast, no fluorescent signal at the injection site was observed in MSC mice (Figures 3A, D arrow).

Fluorescent images of an explanted organ with MSCs and PSL

When fluorescence intensity was measured in explanted organs, intensity was brighter in the brain and testis of both MSCs and PSL administered mice. But fluorescence intensity of PSL was higher than fluorescence intensity of MSC. In MSC mice, increased fluorescence intensity was also found in the liver, omentum, mesentery, kidneys and spleen. In PSL mice, increased fluorescence was also found in the liver, omentum, mesentery, spleen, kidney, lung and adipose tissue around the testis. Fluorescence was barely present in lung and adipose tissue of MSC mice (Figures 3B, C, E, F, G).

Histological identification of transplanted MSCs and PSL

SSEA4 staining was done on brain and testis tissue to identify MSCs and PSL. SSEA4 staining was positive only in the brains and testis of mice administered with PSL. In contrast, SSEA4 staining was negative in MSC mice. As SSEA4 is the marker for human pluripotent stem cells, SSEA4 staining in the brain and testis means that the administered PSLs, which are human pluripotent stem cells, have accumulated in those organs.

Brain and testis tissues of both MSCs and PSL mice were miRNA221-cy5.5 positive. The intensity of miRNA221-cy5.5 in brain tissue was stronger in PSL mice. However, its intensity in testis tissue, was higher in MSC mice (Figures 4A, B).

Discussion

There have been many reports on the efficacy of MSCs and PSCs in treating various diseases[26,27,28,29,1]. Previously, it was believed that PSCs repair damaged tissues through differentiation in injured organs. Therefore, to deliver greater numbers of PSCs to injured tissues, researchers have attempted diverse

injection strategies. The best way to deliver PSCs is intravenous administration. After administration, the PSCs are distributed throughout the body and significant portion presumably reach the damaged organ.

However, there have been few studies tracing and localizing PSCs[30,31]. There has been some reports on the tracing of MSCs in mice. In this study, we tried to trace MSCs and PSL in mice using bioluminescent techniques. The MSCs used were obtained from umbilical cords. These MSCs were identified as positive for CD73, CD90 and CD105 and negative for CD34 and CD45. They also differentiated into adipose cells and chondrocytes. PSLs were induced from MSCs with small molecule compounds (STC-F002) and identified with the human pluripotent markers SSEA4, OCT4 and SOX2. PSL was differentiated into three germ layer cells. Differentiation into ectodermal (neuronal cell), endodermal (hepatocyte) and mesodermal cells (osteoblast and chondrocyte) were demonstrated in this study. PSL has the advantages over other PSCs such as ESCs and iPSCs. The most important problem with ESCs is an ethical concern that the derivation of pluripotent stem cell lines from oocytes and embryos is fraught with disputes. Because of this problem ESCs are rarely used in clinical trials. Another problem is the possibility of malignant transformation. iPSCs also have a problem with becoming cancerous. As iPSCs pluripotency is induced using gene insertion, they also have the potential for malignant transformation. However, the pluripotency of PSL are induced by small molecules without gene insertion and so have no mutational potential. Another advantage of PSL is that massive production is possible due to their high yield rate. CiPSCs are similar to PSL in that their pluripotency is induced by small molecules. However, CiPSCs are still of mouse origin and have a very low production yield rate.

The PSL used in this study are the first PSCs of human origin induced by small molecule compounds. There are reports on administering MSCs to mice. It was been shown there is no interaction between MSC and the immune system of mice[32]. Similarly, human PSL are also expected to have no immunology reaction with mice. When MSCs and PSL were administered to mice, there was no immunologic interaction in this study.

The migration of injected MSCs and PSCs remains unknown. If the migration, localization and fate of this cells are known, the physiologic process of healing or repairing can be studied. There has also been one report on the migration of MSCs in mice. They have shown that MSCs migrate to the lungs, kidneys, and skin of the lower back. In our study, MSCs predominantly migrated to the brain and testis though they also migrated to the liver, omentum, mesentery, kidneys and spleen. Migration of PSL was similar pattern with MSCs in that they went to the brain, testis and other intraperitoneal organs, but PSL migration was more than MSC migration in Brain. This is the first study to show that these cells migrate to the brain and testis. Fluorescent images of explanted organs had higher intensity in the PSL mouse group than the MSC mouse group in the brain. However, in the testis, intensity was higher in the MSC mouse group than the PSL mouse group.

This migration to the brain and testis by PSL was also confirmed by staining for SSEA4, which is a marker for human pluripotent stem cells.

It is unclear why MSCs and PSL predominantly migrate to the brain. There must be some still unknown interaction between these cells and the brain. The amount of stem cells present in the body decreases with age, which may be related to a decrease in organ function[33]. One of the organs susceptible to decreased stem cells might be the brain which may result in dementia[34]. The interaction of PSCs and the brain may be vital for proper brain function.

It is also interesting that MSCs and PSL migrate to the testis. The testis is the locus of reproduction in male animals. The accumulation of these cells in the testis may have some function in reproduction. Reproductive function also decreases with age which is associated with decreased stem cell production.

It is still unknown why stem cell migrates actively into the brain. We presume that brain plays important role in activity of stem cell, although specific activity is should be studied further. The brain uses 25% of oxygen consumed in whole body. And brain uses 50% glucose consumed in whole body. Accordingly, brain may need substantial an amount of regenerative cells like stem cell. After stem cells enter into brain, they may be involved in the function and maintenance of the brain. We do not know why stem cell enters into testis massively either. The testis is organ where cell division is occurring abundantly. Normal cells have limitation of permanent cell division which is not suitable for reproduction. Stem cells have no limitation of cell division which is necessary for characteristic reproductive organ. Reproductive organ is vital for maintenance of the genes for future generation.

These cells also migrate to the liver, kidney, and spleen, which are important organs in homeostasis. These organs are also supplied with large amount of blood which may be related with their active metabolic processes. The accumulation of the cells in these organs is related to metabolism and their own function. Although previous reports on MSCs migration has shown that MSCs accumulate in the lung, there was no accumulation of these cells in the explanted lung in our study. One possible reason is that explanted lung was procured after sacrificing the animals on day 9. The cells present in the lung may have escaped the lung during the later days of the experiment. In a prone *in vivo* image fluorescence was shown around the lung during the initial stage.

It is also interesting these cells accumulated in the omentum and mesentery which invites further study.

In PSL but not MSCs mice, fluorescence was persistent at the injection site in the tail. It is also unknown why this discrepancy occurs. The injection site can be regarded as an injury area. Therefore, persistent fluorescence at the injection site may show the attraction of PSL to the injury site. However, further study of this phenomenon is necessary.

miRNA-fluorescence was used to trace the MSCs and PSL. This technique provides ultra-selective binding to target DNA which can be tracked by optical monitoring of visual imaging. Fluorescence imaging method in this study is very sensitive to detect pico mole of target miRNA with high specificity[35,36,37]. Synthesized molecular beacon conjugated with fluorescent was made for complimentary reaction with miRNA-221. This imaging technique can detect cell localization in *in vivo*[37].

Conclusion

In this study, we found that MSCs and PSL predominantly migrate to the brain and testis. These cells also migrate to the liver, kidney, spleen, mesentery and omentum. The interactions of these cell with each organ are not yet known and further study is necessary to determine their roles. MSC and PSL treatment could be a breakthrough for many incurable diseases. We expect there may be many future studies on these notable treatments.

Declarations

Availability of data and materials

The datasets used and /or analyzed during the current study are available form the corresponding author on reasonable request.

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Contributions

SY, Lee and HS Han contributed to the study design, data acquisition, data analysis, and data interpretation, and drafted and critically revised the manuscript; TH Kim contributed to the study concept and design and data analysis. ST Kim contributed to the study concept, design and drafted and critically revised the manuscript. KH Lee contributed to drafted and critically revised the manuscript. The authors

declare no potential conflicts of interest with respect to the authorship and/or publication of this article. The authors read and approved the final manuscript.

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Ethics declaration

Ethics approval and consent to participate

All experimental programs and materials for animal and human MSC cell experiments in this project met ethical requirements and were reviewed by the Institutional Animal Care and Use Committee and Institutional Review Board of Seoul National University (approval number: BA1604-198/020-01).

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Figures

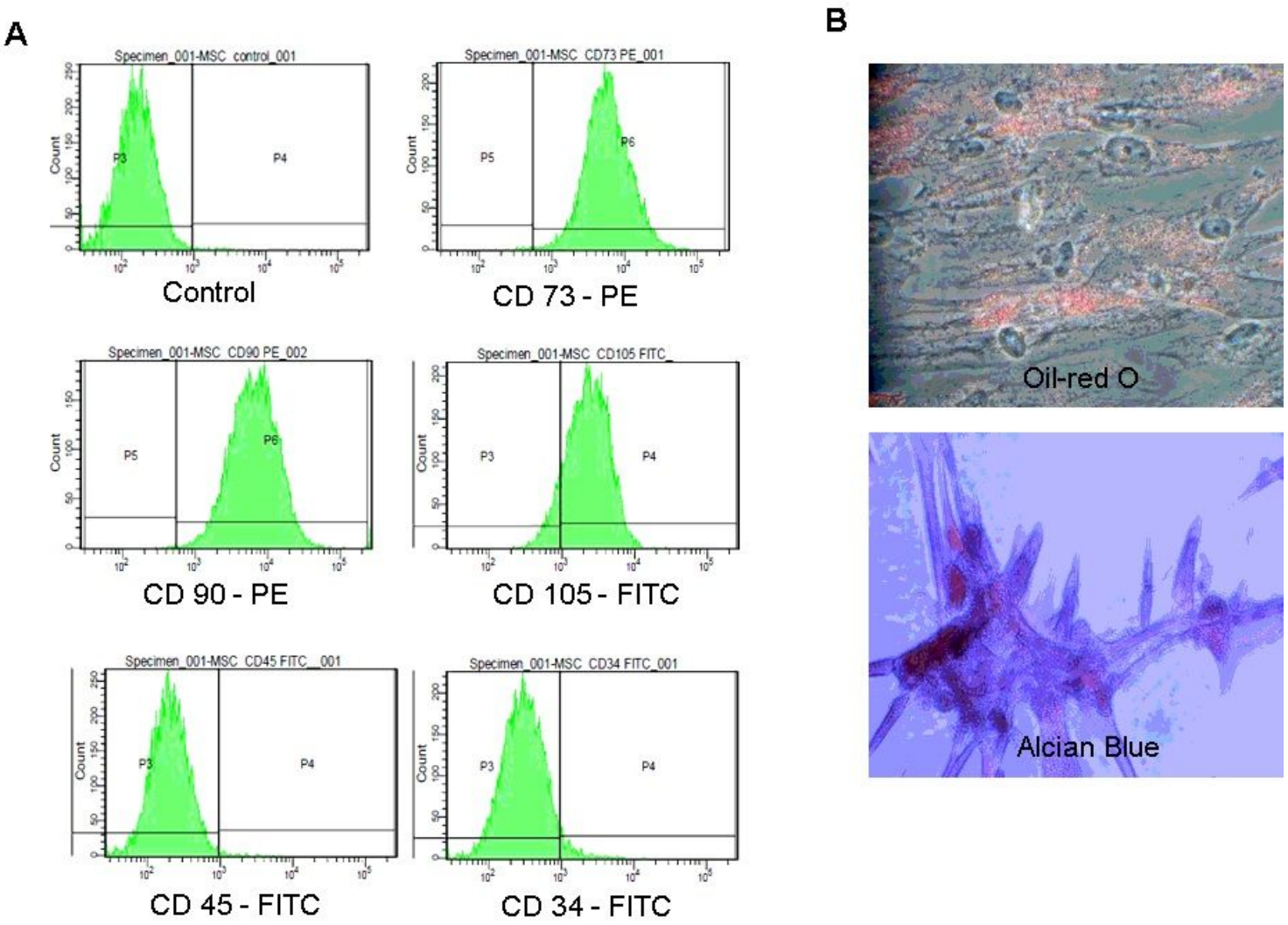

Figure 1

Identification of the adherent cells derived from Umbilical Cords. (A) adherent cells after five passages were found to be positive for CD73 (99.7%), CD90 (99.5%), and CD105 (88.9%) and negative for CD34 (4.0%) and CD45 (0.9%) by flow cytometry. (B)Human UCMSCs treated with adipocytic inductive medium, but not the controls, were stained with Oil Red O. The cells cultured in chodrogenic-inductive medium were stained with Alcian Blue. (The scale bar represents 100 μm).

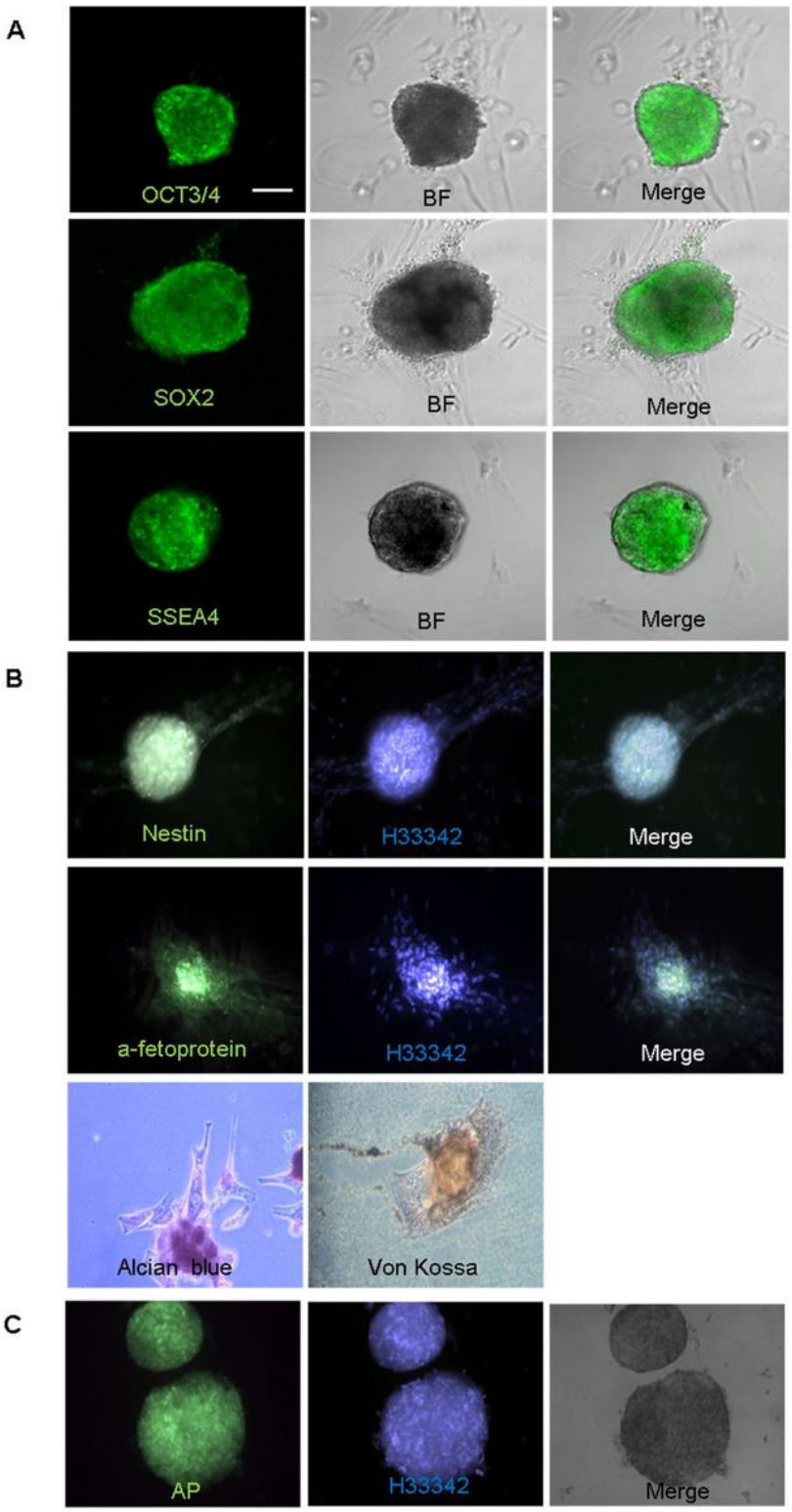

Figure 2

PSL colonies express pluripotent stem cell markers and differentiate in ectodermal, endodermal and mesodermal cells. (A) Immunofluorescence microscopy demonstrates that PSL cell colonies and individual PSL cells express characteristic pluripotent stem cell markers, including SSEA4, Sox2 and Oct3/4. Nuclei were stained with H33342 (blue). Original magnification, 200 X. (The scale bar represents 100 μm). (B)PSL cells differentiated into neuronal cells (nestin) as ectodermal cells and hepatocytes

(alpha-fetoprotein) as endodermal. PSL cells were also differentiated into chondrocyte (alcialn blue) and osteoblast (Von Kossa) mesodermal cells. Nuclei were stained with H33342 (blue). Original magnification was 200 X. (C) PSL cell colonies express characteristic pluripotent stem cell markers of Alkaline Phosphate. Nuclei were stained with DAPI (blue). Original magnification, 200 X.

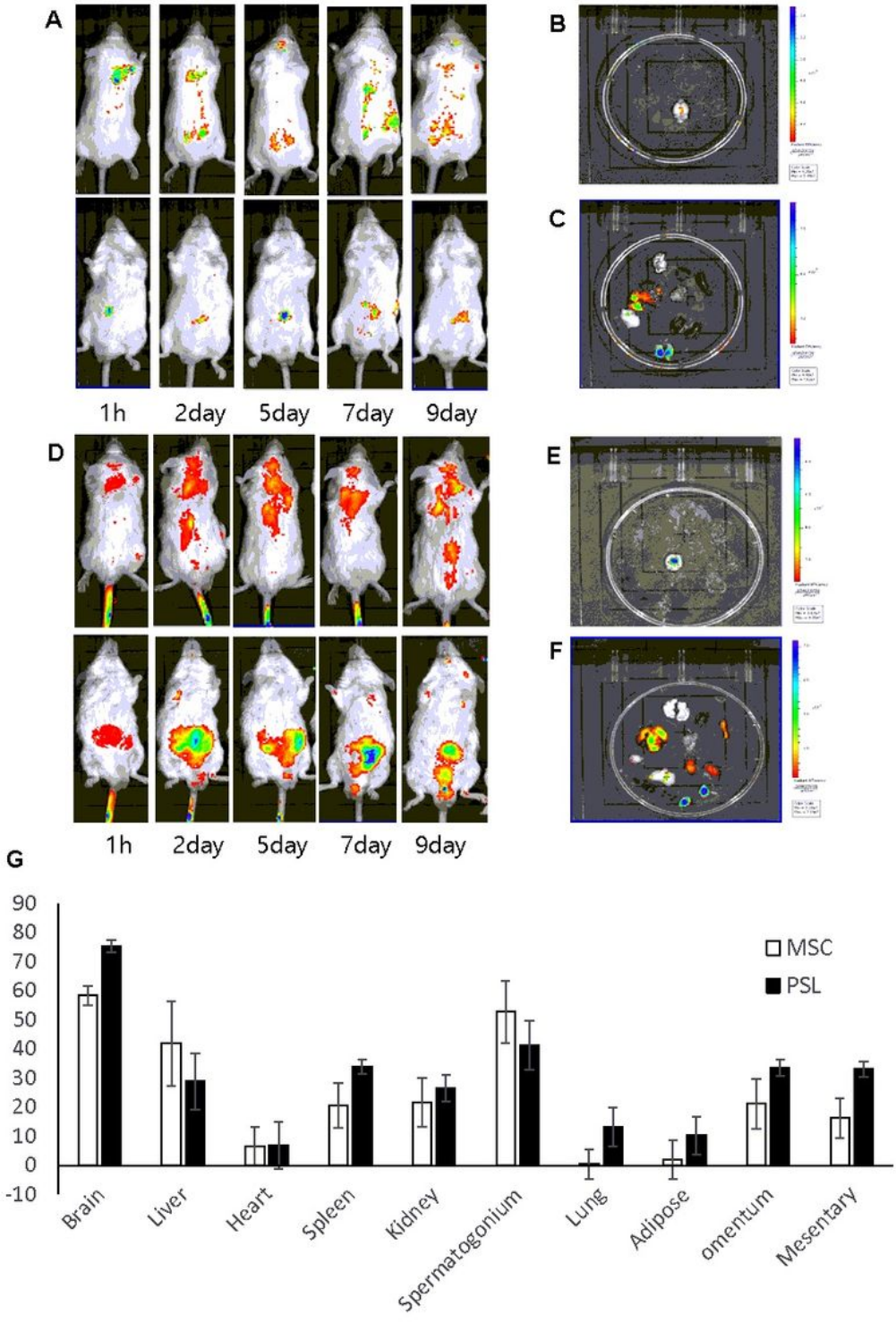

Figure 3

Ex vivo images showing the distribution of fluorescent cells in different organs as detected by the indocyanine green filter of the IVIS. (A) IVIS image of the transplanted miRNA221-Cy5.5-MSCs in vivo. Quantitative analysis of IVIS image signals in the chest and back. P < 0.05 versus IVIS signals in the image (n = 9); #P < 0.05 versus FI signals in the back at 1.5 h (n = 9). (B) IVIS photographs of dissected brain with MSCs. (C) IVIS photographs of dissected liver, heart, spleen, kidney, testis, lung, adipose tissue, omentum and intestine mesentery with MSCs. (D) IVIS image of transplanted miRNA221-Cy5.5-PSL in vivo Quantitative analysis of IVIS image signals in the chest and back. Arrow is highly fluorescence intensity in injection site. P < 0.05 versus IVIS signals in the image (n = 9); #P < 0.05 versus IVIS signals in the back at 1.5 h (n = 9). (E) IVIS photographs of dissected brain with PSL. (F) IVIS photographs of dissected liver, heart, spleen, kidney, testis, lung, adipose tissue, omentum and intestine mesentery with PSL. (G). A graph showing the highest uptake of MSC and PSL cells in the brain compared with the migration of MSC and PSL in various organs.

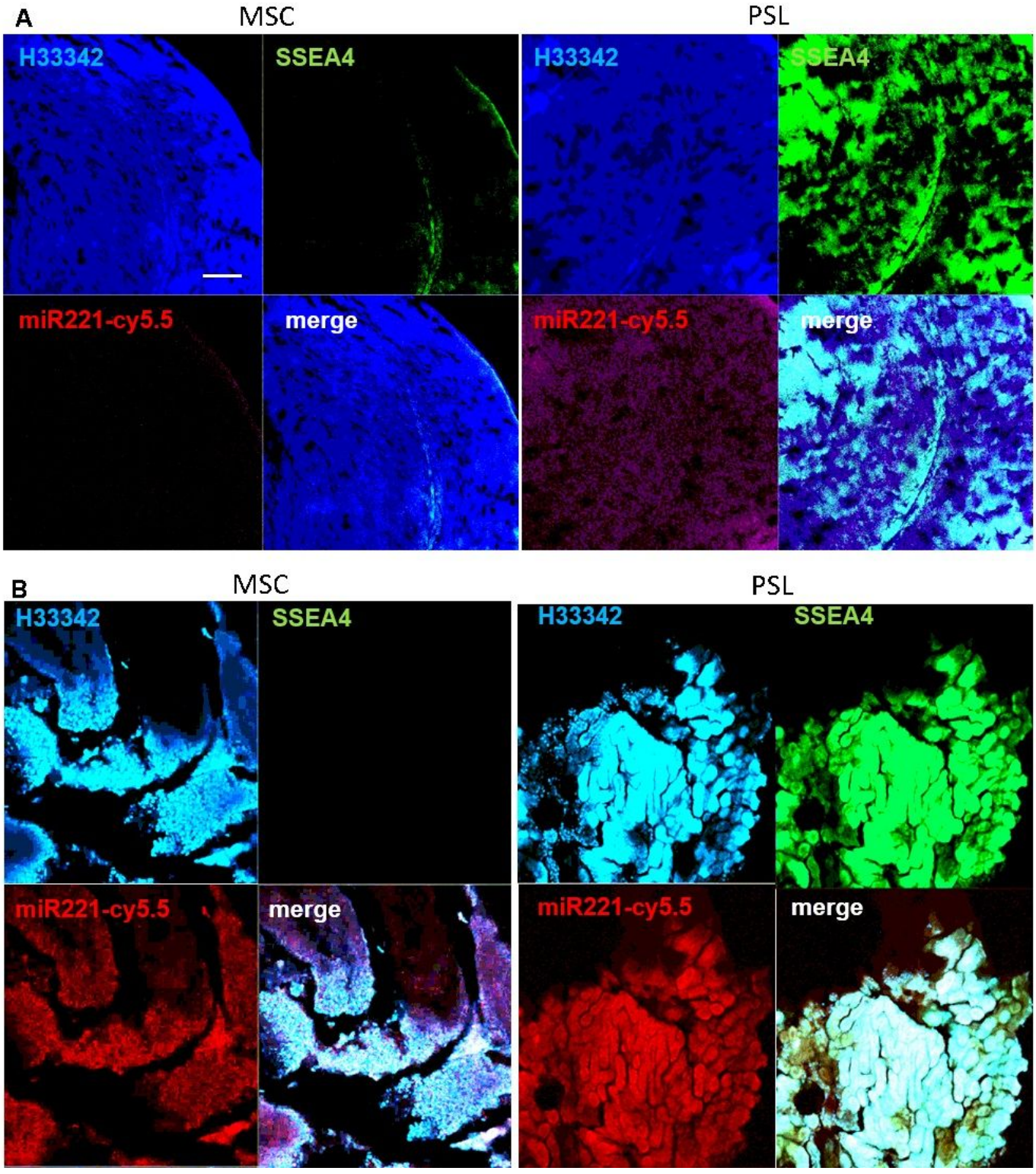

Figure 4

Detection of miRNA221-cy5.5 MSC and PSL in Brain and testis using fluorescence. (A) Fluorescence images of MSC and PSL in the brain stained for SSEA4 (green), miRNA221-cy5.5 (red), and nuclei (blue). Insets: Magnified views of representative fusion products. Scale bar = 100 μm. (B) Fluorescence images of MSC and PSL in testis stained for SSEA4 (green), miRNA221-cy5.5 (red), and nuclei (blue). (Zeiss ZEN lite blue 2.5 software: https://www.zeiss.com/microscopy/int/downloads.html)